Review

Platelets in neutrophil recruitment to sites of inflammation

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Abstract

Purpose of review: This review describes the essential roles of platelets in neutrophil recruitment from the blood stream into inflamed and infected tissues, with a focus on recent findings.

Recent findings: Platelets are required for the recruitment of neutrophils to sites of inflammation and infection. They fulfil this role largely by enabling contacts of circulating neutrophils with the inflamed blood vessel wall prior to extravasation. Platelets promote both early stages of neutrophil recruitment (tethering, rolling, arrest, firm adhesion) and - as recent work has demonstrated – later stages (intravascular crawling and diapedesis). Recent studies have also begun to identify platelet signaling pathways that can elicit the underlying interactions between platelets, neutrophils and vascular endothelial cells without stimulating concomitant platelet aggregation and thrombus formation. These pathways include Rho-GTPases and Rho-GEFs.

Summary: Recent findings have contributed to our burgeoning understanding of the platelet-dependent mechanisms that control neutrophil recruitment to sites of inflammation and have opened up new avenues of research aimed at increasing our knowledge of these mechanism further. These insights might lead to the development of novel anti-inflammatory drugs that will be useful in a wide range of inflammatory diseases without causing immunodeficiency.

Keywords

Platelets, neutrophils, guanine-nucleotide exchange factors (GEFs), P-Rex1, Vav, P-Selectin Glycoprotein Ligand-1 (PSGL-1), P-selectin.

Introduction

Neutrophils are rapidly recruited from the blood stream into inflamed and infected tissues, where they release proinflammatory chemokines and cytokines to attract other inflammatory cells and where they mount effector functions to clear bacterial and fungal infections [1-3]. The importance of neutrophil recruitment and neutrophil effector functions is evident in Human Leukocyte Adhesion Deficiency disorders and in Neutrophil Immunodeficiency Syndrome, which are characterized by severe recurrent infections [4, 5]. Thus, neutrophil recruitment is clearly crucial for innate immunity. However, it must be tightly regulated, as excessive recruitment can lead to inflammatory disorders, tissue destruction and loss of organ function [1-3].

Over recent years, platelets have become recognized as essential regulators of the recruitment and effector functions of many types of leukocytes, including neutrophils, under a wide range of inflammatory conditions. Importantly, these pro-inflammatory roles of platelets are distinct from their well-understood role in hemostasis (blood clotting), which is largely unaffected during inflammation. The various roles of platelets during inflammation have been reviewed extensively over the past 18 months [6-11]. Here we focus on neutrophil recruitment.

Studies using methods to deplete platelet levels or to inhibit the interactions of platelets with circulating neutrophils and with vascular endothelial cells have shown that platelets are required for neutrophil recruitment in many acute and chronic inflammatory situations, including acute septic, aseptic or allergic pulmonary inflammation [12-19], peritonitis [15], pancreatitis [20], atherosclerosis [21], rheumatoid arthritis [22] and encephalomyelitis (multiple sclerosis) [23].

Platelet-dependent cell interactions in neutrophil recruitment

Neutrophil recruitment from the circulation into inflamed and infected tissues proceeds in well-defined steps. Neutrophils are initially captured from the fast-flowing blood stream in the

postcapillary microvasculature by selectin-dependent loose tethering to the endothelial vessel wall, followed by rolling along the endothelium, integrin-dependent arrest and firm adhesion, intravascular crawling and trans-endothelial migration into the inflamed tissue [1]. There is evidence that platelets promote all steps of this process from tethering to transmigration (**Figure 1**).

Inflammatory stimuli induce the upregulation of adhesion molecules on the surface of platelets and vascular endothelial cells that enables these cells to stick to each other and to neutrophils, thus building physical bridges that vastly increase the efficiency of the tethering, rolling and adhesion of neutrophils on the vascular endothelium. In addition to these physical contacts, platelets stimulate adhesion-molecule dependent signaling in neutrophils and vascular endothelial cells, and secrete soluble factors which further promote neutrophil adhesion.

A highly significant adhesion molecule for platelet-neutrophil interactions is P-selectin on the platelet surface [24] which binds P-Selectin Glycoprotein Ligand 1 (PSGL-1) on the neutrophil surface. The importance of platelet P-selectin was determined by the use of P-selectin deficient mice or blocking antibodies for P-selectin and PSGL-1 [13, 25-32] which showed that this interaction is crucial for the interactions of neutrophils with the vascular endothelium from the earliest stages of recruitment (tethering, slings) through to transmigration (please also see reference [33] for a recent review). Binding of platelet P-selectin to neutrophil PSGL-1 induces inside-out signaling that causes neutrophil $\beta 2$ integrins to adopt their open conformation [34-36]. Furthermore, a recent landmark publication showed that platelet P-selectin dependent neutrophil PSGL-1 signaling must be spatially restricted to the neutrophil uropod to ensure the correct localization of the chemokine receptors and integrins that control neutrophil intravascular adhesion and crawling [32].

In addition to platelet P-selectin/neutrophil PSGL-1, various integrin- and glycoprotein dependent interactions contribute to platelet-neutrophil interactions. For example, platelet intercellular adhesion molecule-2 (ICAM-2) binds the neutrophil integrin leukocyte function antigen-1 (LFA-1, CD11a/CD18), platelet glycoprotein GPIb binds the neutrophil integrin Macrophage-1

Antigen (Mac-1, complement receptor 3, CR3, CD11b/CD18), and platelet CD40L interacts with neutrophil CD40, a protein of the TNF-receptor superfamily. Platelets also express PSGL-1 [37], and it seems likely that binding of neutrophil L-selectin to this platelet PSGL-1 also contributes to platelet-neutrophil interactions, but that remains to be investigated. Moreover, platelets and neutrophils can bind indirectly to each other using extracellular matrix proteins as bridges, for example both the platelet glycoprotein integrin $\alpha_{IIb}\beta_3$ (GPIIb/IIIa) and neutrophil Mac-1 can bind to fibrinogen (please see also reference [33] and references therein).

Similarly, platelet attachment to the endothelium (which can be extensive, with platelets effectively "carpeting" the vessel wall) is conferred by endothelial P-selectin (and possibly also E-selectin) to platelet PSGL-1 [37], as well as by glycoprotein and integrin-dependent interactions such as platelet GPIbα or GPIIb/IIIa binding to endothelial von Willebrand factor (vWF) or ICAM-1 [38-40]. Endothelial P-selectin is stored on Weibel-Palade bodies which are acutely upregulated in response to inflammatory mediators such as tumor necrosis factor-α (TNFα), and platelets can also induce this upregulation, by secreting serotonin (5-HT) [41, 42]. A recent report showed that platelet adhesion to the vascular endothelium occurs predominantly at endothelial cell junctions, and that these junctional platelets not only facilitate neutrophil capture in a P-selectin dependent manner, but actually guide rolling and crawling neutrophils to their site of transmigration, which was believed to be largely a matter of chance up to now, with neutrophils happening upon weakened endothelial cell/cell junctions [40].

Among the platelet ligands that contribute to interactions with integrins on neutrophils and vascular endothelial cells are ICAM-2 [43], various junctional adhesion molecules (JAMs) and platelet endothelial cell adhesion molecule-1 (PECAM-1). These platelet JAMs and PECAM-1 are required for neutrophil transendothelial migration, at least in part by modulating the strength of the endothelial cell/cell interactions that govern the barrier function of the vasculature [10, 44, 45].

It should be noted that the types of adhesion molecules that confer physical interactions between platelets, neutrophils and vascular endothelial cells vary between inflamed tissues and organs. For example, neutrophil recruitment to the lung seems to be more dependent on PSGL-1 than on P-selectin, unlike in other tissues, suggesting that PSGL-1 interactions with other types of selectins, such as L- and E-selectin, may important during pulmonary inflammation [13, 33].

Activated platelets release a host of chemokines that can activate neutrophils, including platelet factor 4 (PF4, CXCL4), RANTES (CCL5), neutrophil-activating peptide-2 (NAP-2, CXCL7) and the CXCR2 ligand platelet basic protein (PBP). Both PF4 and NAP-2 stimulate neutrophil endothelial adhesion, and NAP-2 also stimulates neutrophil chemokinesis, chemotaxis and trans-endothelial migration, which are important during recruitment steps following neutrophil arrest [33, 46]. This ability of platelets to stimulate neutrophil migration is P-selectin dependent and can be induced by the activation of platelets through factors that do not stimulate neutrophil migration directly, such as the chemokines MDC (CCL22) and TARC (CCL17) [21]. Accordingly, platelet-derived RANTES and NAP-2 were shown to be required for neutrophil recruitment during lipopolysaccharide (LPS)-induced acute lung injury (ALI) [47]. NAP-2 derived from thrombi of platelets on the vessel wall was also recently found to induce intravascular neutrophil chemotaxis towards the thrombus during vascular injury [48].

Proinflammatory platelets also induce the release of chemokines from vascular endothelial cells, through the secretion of storage vesicles, including interleukin-8 (IL-8, CXCL8) from Weibel-Palade bodies. Rather than disappearing into the circulation, secreted IL-8 remains immobilized on the endothelial surface and stimulates the GPCR CXCR1 on neutrophils [49]. This leads to the integrin Mac-1 on the neutrophil surface adopting its open, active conformation through inside-out signaling, thus stimulating firm neutrophil adhesion, intravascular crawling and transmigration [50].

Moreover, it has recently been demonstrated that soluble platelet-derived factors can also control neutrophil extravasation at a step after transmigration, at least in the lung. Signaling through

the Wnt GPCR signaling pathway inhibits the expression of ICAM-1 and VCAM-1 on the surface of alveolar epithelial cells, and platelets were shown to secrete the Wnt antagonist Dickkopf-1 (Dkk1) to block this pathway, thus allowing neutrophils undergoing diapedesis to adhere to these epithelial cells and be recruited into the lung during ALI [18].

It remains to be seen if similar platelet-dependent pathways operate in other tissues to stimulate neutrophil recruitment steps beyond diapedesis, or whether this is a characteristic feature of lung alveolae, where endothelial and epithelial cells are closely adjacent without much interstitium to separate them. Platelets, or microparticles derived from platelets, can certainly be observed in many inflamed tissues [6, 22, 23, 51], but it remains unclear how they get there. They might be piggy-backing on neutrophils, be flushed passively though a leaky endothelium, or be actively migrating by chemotaxis. Support for the second possibility comes from the finding that platelet microparticles, small enough to pass through endothelial cell/cell junctions, are present in synovial fluid of rheumatoid arthritis patients [22, 52]. Support for the third possibility comes from studies reporting chemotaxis of isolated platelets in response to the stimulation of CXCR4 by stromal-derived factor-1 (SDF-1) in a PI3K-dependent manner [53], and in response to allergen after prior in vivo sensitization of mice with ovalbumin [51]. In general though, the fate of platelet-neutrophil complexes beyond the vasculature and the importance of platelet-derived soluble mediators in stimulating interstitial neutrophil migration towards sources of inflammation and infection still requires much investigation.

Platelet dependent signalling pathways that control neutrophil recruitment

While the surface molecules through which platelets interact with neutrophils and vascular endothelial cells to mediate neutrophil recruitment are now quite well-understood, the platelet

signaling pathways that elicit these interactions without causing concomitant platelet aggregation and blood clotting are still quite unclear.

Platelet P-selectin is stored on the membrane of α-granules under basal conditions. Its upregulation onto the platelet surface is one of the earliest events during neutrophil recruitment and is required for platelet-neutrophil interactions. This upregulation occurs through the degranulation of platelet α-granules in response diverse stimuli, including inflammatory mediators such as chemokines, immunoglobulins and factors derived from bacteria (e.g. LPS, a component of the outer membrane of gram-negative bacteria such as Escherichia coli) [33]. However, degranulation occurs in response to pro-thrombotic platelet agonists such as thrombin, and is thus not a means of generating pro-inflammatory platelets specifically.

Adhesion molecule-dependent pathways are elicited through the physical interactions of platelets with neutrophils and vascular endothelial cells. Platelet PSGL-1 signaling has not been studied yet in detail, but by analogy with PSGL-1 signaling pathways in other cell types [36, 37, 50], we can infer that the engagement of platelet PSGL-1 by neutrophil or endothelial selectins is likely to induce ITAM-dependent signaling through Src, Syk and Btk family protein tyrosine kinases, phosphoinositide 3-kinase (PI3K), phospholipase C and mitogen activated kinases (MAPKs), and that this would result in the upregulation and open conformation of platelet integrins.

Activation of platelet integrins by neutrophil and endothelial integrin ligands induces signal transduction through protein tyrosine kinases, PI3K, Rap1, Rho-GEFs and Rho-GTPases (see below), among other effectors, to stimulate platelet adhesion and spreading [54, 55]. However, as with P-selectin, integrin signaling is not specific to proinflammatory platelets, it also promotes aggregation and thrombus formation [54]. For example, during bleeding, platelets bind to collagen on the extraluminal side of the blood vessel exposed by tissue injury through $\alpha_2\beta_1$ (GP1a/IIa, VLA-2) (and indirectly through GPIIb/IIIa, via von vWF) [56], and during blood clotting they bind to fibrinogen and fibrin via $\alpha_{IIb}\beta_3$ [57]. Thus, the pro-inflammatory versus pro-thrombotic outcome of platelet integrin

signaling depends on the type of integrin ligands they are exposed to, as well as on crosstalk with other pathways elicited by the presence of inflammatory stimuli.

It is increasingly becoming apparent that, in addition to adhesion molecule-dependent pathways, toll-like receptor (TLR) and G protein coupled receptor (GPCR) signaling pathways are important for proinflammatory platelet functions [6, 7, 58]. Platelets express various TLRs (including TLR1, 2, 4 and 9) which recognize pathogen-associated molecular patterns (PAMPs) [33]. Signaling through the LPS receptor, TLR4, is emerging as particularly important for platelet-dependent neutrophil recruitment, as it can induce platelet-neutrophil interactions without causing platelet aggregation (although it increases aggregation induced by other stimuli) [59-61]. In nucleated cells, TLR4 signals through the adaptor protein Myd88, the IRAK kinases, TRAF proteins, Akt and Jnk to induce nuclear events (e.g. NFκB dependent transcription) [62], and at least the cytoplasmic part of this pathway also operates in platelets [63, 64]. However, a recent study using platelet-specific deletion of MyD88 suggested that platelet MyD88 is dispensable for pulmonary neutrophil recruitment and the development inflammation during Klebsiella-induced septic pneumonia [65]. This raises the possibility that TLR4 signaling through Myd88-independent pathways (perhaps cGMP [63]) may be more important during platelet-dependent neutrophil recruitment.

Among platelet GPCR pathways, signaling through thrombin receptors PAR1 and PAR4 is the archetypical pro-thrombotic pathway, where the protease thrombin cleaves the N-terminus off the receptor to generate an active GPCR [66]. Yet, even that pathway has recently been implicated in neutrophil recruitment, as thrombin-dependent cleavage of platelet PAR4 was shown to induce neutrophil recruitment to sites of vascular injury [67]. Furthermore, the PI3K target Akt2 was recently shown to be required for neutrophil-platelet interactions during vascular inflammation. Inhibition or deficiency of Akt2 in platelets was sufficient to impair the interaction of thrombin-stimulated platelets with neutrophils, suggesting that Akt2 is an important effector of thrombin signaling during vascular inflammation [68].

In comparison to thrombin, the GPCR ligand ADP induces a weaker pro-thrombotic signal, and signaling through the P2Y1 ADP-receptor and its downstream target RhoA was recently shown to be required for platelet-neutrophil interactions and for neutrophil recruitment to the lung during allergic inflammation [69].

In addition to adhesion-molecule and receptor-dependent pathways, proinflammatory platelet functions are also known to be enhanced by extracellular acidosis, a characteristic feature of tissue injury, whereas the hemostatic functions of platelets are decreased by acidosis [33, 70]. However, the molecular basis of this preferential stimulation of the inflammatory functions of platelets under low pH conditions is still unknown.

Platelet Rho-GTPases and Rho-GEFs in neutrophil recruitment

The Rho-family small GTPases Rac1 and RhoA [71] are expressed in platelets and control actomyosin cytoskeletal dynamics, and thus platelet morphology, adhesion and degranulation [72-75]. Recent studies using the Rac1 inhibitor NSC23766 showed that Rac1 activity is required for the secretion of RANTES and PF4 from platelets by regulating the degranulation of α-granules, and that these platelet-derived factors are required for neutrophil recruitment to the lung during bacterial infections [16, 17]. Similarly, the importance of platelet RhoA activity was recently demonstrated by the inability of platelets treated with an inhibitor of the RhoA target ROCK to reconstitute neutrophil recruitment in thrombocytopenic mice during allergic pulmonary inflammation [69].

Rho-GTPases are activated by guanine-nucleotide exchange factors (GEFs) [76]. The Rac-GEFs P-Rex1, Vav1 and Vav3 are known to be expressed in platelets and to play minor roles in platelet aggregation and hemostasis [75, 77, 78]. P-Rex1, which is activated by PIP_3 (the lipid second messenger produced by PI3K) and the G$\beta\gamma$ subunits of heterotrimeric G proteins, controls neutrophil recruitment through both neutrophil-intrinsic and –extrinsic mechanism [79-82]. Neutrophil-intrinsic

roles include E-selectin and LFA-1-dependent rolling on vascular endothelium and Mac-1-dependent intravascular crawling [81], whereas neutrophil-extrinsic roles comprise the regulation of vascular permeability through E-cadherin endothelial cell junctions [83]. Vav1 and Vav3, which are activated by tyrosine kinases in response to the stimulation of various receptors such as integrins, Fc receptors and GPCRs [84], alone are dispensable for neutrophil recruitment, but do cooperate with P-Rex1 to regulate neutrophil recruitment and effector functions [15, 72, 85]. Neutrophil transmigration and airway infiltration are all-but-lost in Prex1$^{-/-}$Vav1$^{-/-}$ and Prex1$^{-/-}$Vav3$^{-/-}$ mice during LPS-induced pulmonary inflammation, due to an impairment in L- and E-selectin dependent neutrophil adhesion to airway postcapillary venules and in ICAM-1 dependent slow rolling [15].

Importantly, this crucial role of the two Rac-GEF families in neutrophil recruitment was dependent on their expression in platelets. Unlike wild-type platelets, Prex1$^{-/-}$Vav1$^{-/-}$ or Prex1$^{-/-}$ Vav3$^{-/-}$platelets failed to reconstitute neutrophil recruitment in thrombocytopenic mice, both during acute septic and allergic pulmonary inflammation [15]. The severity of the phenotype was similar to that seen in Prex1$^{-/-}$Vav1$^{-/-}$ and Prex1$^{-/-}$Vav3$^{-/-}$ mice, which suggested that the expression of these Rac-GEFs in platelets is more important for neutrophil recruitment than their neutrophil-intrinsic roles. P-selectin levels were normal on the surface of Prex1$^{-/-}$Vav1$^{-/-}$ and Prex1$^{-/-}$Vav3$^{-/-}$ platelets, but PSGL-1 levels were reduced, as was the incidence of platelet-neutrophil complexes in the circulation. Thus, the loss of L- and E-selectin dependent neutrophil adhesion to the airway microvasculature seen in Prex1$^{-/-}$Vav1$^{-/-}$ and Prex1$^{-/-}$Vav3$^{-/-}$ was likely caused by impaired interactions of neutrophils with these platelets, due to the reduced surface levels of platelet PSGL-1 [15] (**Figure 2**).

Finally, the importance of platelet P-Rex and Vav Rac-GEFs was not restricted to neutrophil recruitment. The pulmonary recruitment of eosinophils, monocytes and lymphocytes was equally compromised in P-Rex1$^{-/-}$Vav1$^{-/-}$ and P-Rex1$^{-/-}$Vav3$^{-/-}$ mice during allergic inflammation in a platelet-dependent manner [15]. Airway inflammation was essentially abolished in these mice,

resulting in improved airway responsiveness [15]. Therefore, platelet P-Rex and Vav family Rac-GEFs play widely important roles in leukocyte recruitment to sites of inflammation. This preferential role in proinflammatory rather than hemostatic platelet functions implies possibilities for novel therapeutic approaches in the treatment of inflammatory disorders.

Conclusion

It should be re-iterated that neutrophil recruitment is only one of the many pro-inflammatory functions of platelets. Platelets are also important for neutrophil effector functions such as reactive oxygen species (ROS) formation, phagocytosis and neutrophil-extracellular traps (NETs) formation, as well as for the recruitment and effector functions of many other types of leukocytes which have recently been reviewed in detail elsewhere [6-9].

The importance of P-selectin and PSGL-1 for platelet-neutrophil interactions during neutrophil recruitment suggests that targeting these molecules might be an effective strategy for novel anti-inflammatory therapies. Accordingly, several drugs that inhibit the synthesis or function of P-selectin or PSGL-1, including small molecule compounds (Bimosiamose) and blocking antibodies (SelK1, SelG1) are in clinical trials for the treatment of inflammatory disorders such as Crohn's disease, COPD, asthma and psoriasis [86]. Furthermore, compounds that inhibit the synthesis of PSGL-1 under inflammatory conditions, rather than affecting expression at resting state are being developed, with the hope that future drugs will target the requisite role of selectins during leukocyte recruitment without affecting the necessary immune-surveillance of the host [87]. Finally, other experimental drugs, such as a non-anticoagulant form of heparin, have recently been reported to disrupt platelet- dependent neutrophil recruitment in animal models [88]. In the future, it may thus possible to use similar therapeutic strategies to specifically target the formation of platelet-dependent inflammatory disorders without affecting the hemostatic functions of platelets.

Key points

- During inflammation, platelets enable neutrophil capture from the blood stream, rolling and adhesion on the vascular endothelium, intravascular crawling and transmigration.
- Rho-GTPases and Rho-GEFs control the proinflammatory responses of platelets required for neutrophil recruitment during inflammation.
- P-Rex and Vav family Rac-GEFs in platelets are required for the recruitment of neutrophils to the inflamed lung by enabling L- and E-selectin dependent neutrophil adhesion to the pulmonary vascular endothelium.

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Abbreviations

ALI, acute lung injury. Dkk1, Dickkopf-1. GEF, guanine-nucleotide exchange factor. GPCR, G protein coupled receptor. ICAM, intercellular adhesion molecule. IL-8, interleukin-8. JAMs, junctional adhesion molecules. LAD, leukocyte adhesion deficiency. LFA-1, leukocyte function antigen-1. LPS, lipopolysccharide. Mac-1, Macrophage-1 Antigen. MAPK, mitogen activated kinase. NAP-2, neutrophil-activating peptide-2. PBP, platelet basic protein. PECAM-1, platelet endothelial cell

adhesion molecule-1. PI3K, phosphoinositide 3-kinase. PIP_3, phosphatidyl-inositol (3,4,5) trisphosphate. PF4, platelet factor 4. P-Rex, PIP_3-dependent Rac exchanger. PSGL-1, P-selectin glycoprotein ligand-1. ROCK, Rho-associated protein kinase. RTK, receptor tyrosine kinase. GTPase, guanine-nucleotide binding protein. SDF-1, stromal-derived factor-1. TNFα, tumor necrosis factor-α. TLR, toll-like receptor. vWF, von Willebrand factor.

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***** This paper showed that a non-anticoagulant form of heparin can be used to inhibit platelet-dependent neutrophil recruitment to the lung during pulmonary inflammation in mice. It acts by inhibiting diapedesis, likely by reducing the level of P-selectin on the platelet surface.

Figure Legends

Figure 1. Platelets in neutrophil recruitment

Neutrophil recruitment from the blood stream into inflamed and infected tissues begins with the upregulation of P-selectin on the surface of vascular endothelial cells and circulating platelets in response to inflammatory stimuli such as TNFα and LPS. Binding of platelet P-selectin to neutrophil PSGL-1 confers platelet-neutrophil interactions **(1)** which are strengthened by the engagement pf additional adhesion molecules such as integrins and glycoproteins **(2)**. Similar adhesion molecules confer interactions between vascular endothelial cells and platelets, including endothelial P-selectin binding to platelet PSGL-1 **(3)**. Physical contacts between all three cell types enable neutrophil tethering and rolling along the vascular endothelium [note that some of the depicted contacts, namely the binding of neutrophil L-selectin and endothelial E-selectin to platelet PSGL-1, are hypothetical; see Figure 2] **(4)**. These interactions, together with neutrophil GPCR signaling, induce the upregulation and opening of the neutrophil integrins LFA-1 and Mac-1. Active LFA-1 confers neutrophil arrest and Mac-1 firm adhesion and intravascular crawling **(5)**. Signaling through platelet adhesion molecules, TLRs and GPCRs promotes these processes by leading to further cell/cell interactions and to the secretion of soluble factors such as chemokines that activate neutrophils and vascular endothelial cells. Moreover, platelets were recently shown to adhere to the vascular endothelium preferentially at endothelial cell/cell junctions and to actively guide neutrophils to the site of trans-endothelial migration **(6)**.

Figure 2. Platelet Rac-GEFs in neutrophil recruitment

Expression of the Rac-GEFs P-Rex1, Vav1 and Vav3 in platelets controls PSGL-1 levels on the platelet surface as well as platelet-neutrophil interactions in the circulation. Prex1/Vav1 or Prex1/Vav3-deficiency in platelets is sufficient to block neutrophil recruitment during septic and allergic

pulmonary inflammation by preventing neutrophil adhesion to the airway microvasculature, which is L- and E-selectin dependent. Mechanistically, the lack of PSGL-1 on the surface of Prex1/Vav-deficient platelets could cause the loss of L- and E-selectin dependent neutrophil adhesion if, for example, PSGL-1 on the platelet surface were a ligand for neutrophil L-selectin and endothelial E-selectin in the airway vasculature. A lack of these PSGL-1/selectin interactions would limit further cell/cell contacts by preventing integrin upregulation and activation. Importantly, despite the key roles of platelet P-Rex1 and Vav Rac-GEFs in neutrophil recruitment, Prex1/Vav-deficient platelets undergo normal aggregation in response to thrombin or ADP. Hence, P-Rex1 and Vav Rac-GEFs seem to preferentially control pro-inflammatory rather than pro-thrombotic platelet functions.

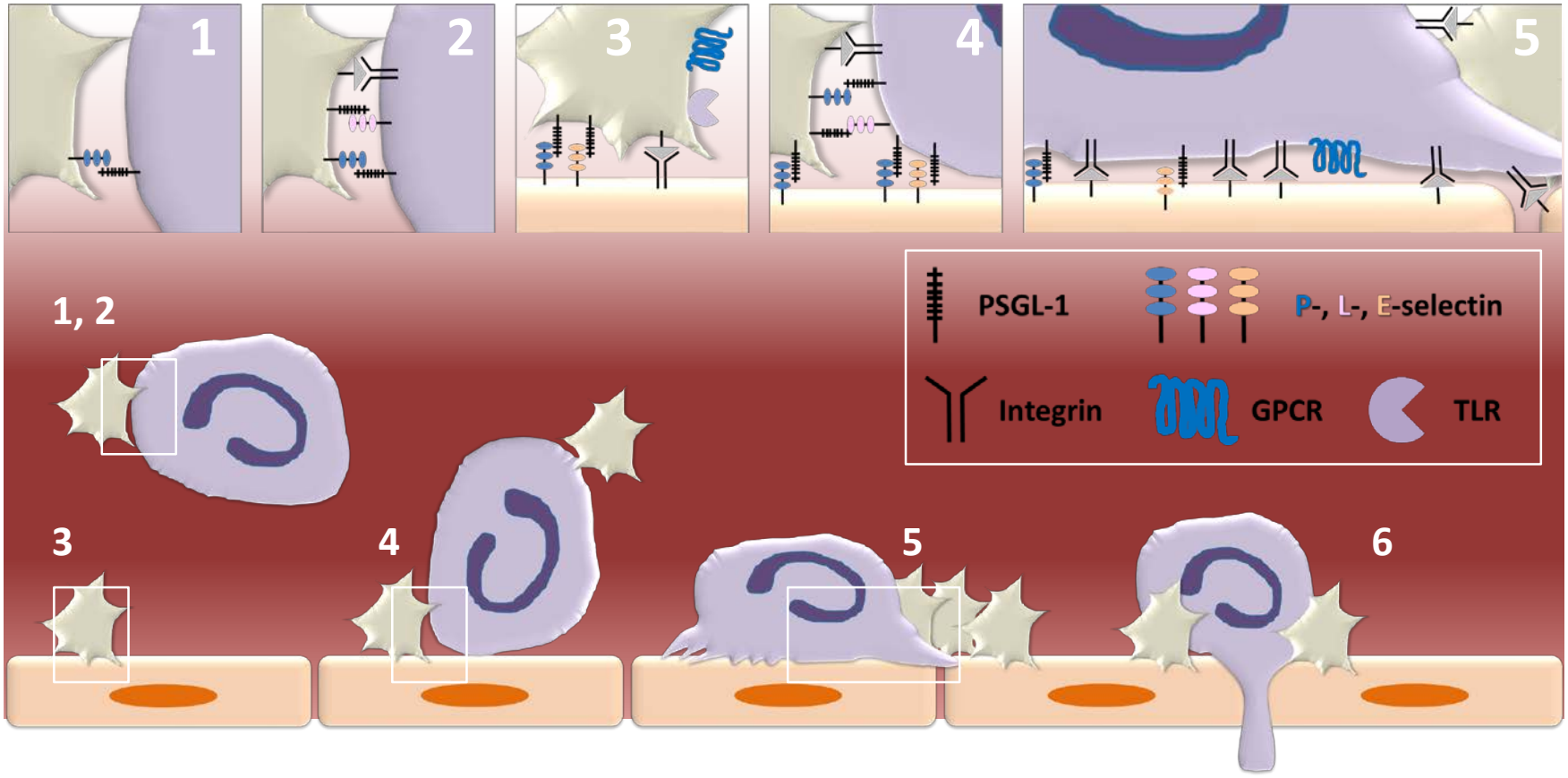

Figure 1, Pitchford et al

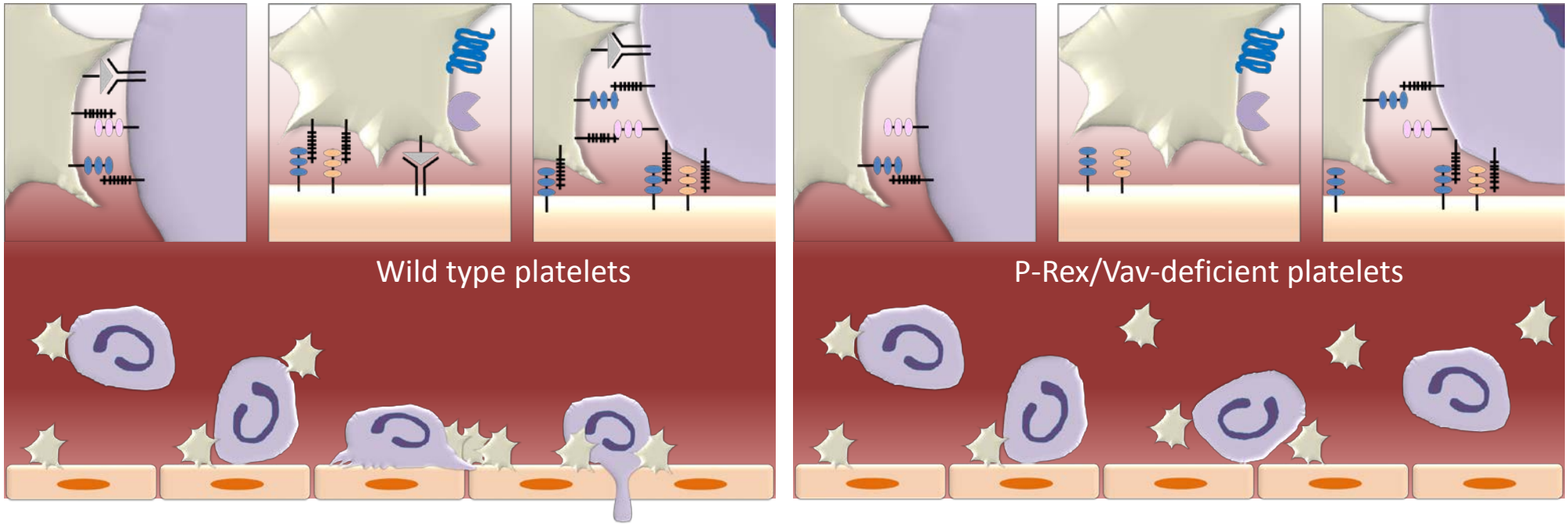

Figure 2, Pitchford et al